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Management of a Patient With a Mechanical Aortic Valve and Antibodies to Both Thrombin and Factor V After Repeat Exposure to Fibrin Sealant

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We describe a patient who developed a markedly prolonged PT, PTT, and thrombin time 13 days after repeat exposure to fibrin sealant during coronary artery bypass grafting and aortic valve replacement. Evaluation revealed an inhibitor to bovine thrombin that cross-reacted with human thrombin. In addition an inhibitor to human coagulation factor V was identified. Despite coagulation abnormalities there was no evidence of bleeding. Nevertheless, effective anticoagulation was required to minimize the thrombotic complications associated with the patient's prosthetic valve. We elected to take a conservative approach and not utilize pharmacologic anticoagulation until there was diminution in the effect of the acquired inhibitors. We report on our patient's course and review the available literature addressing the management of patients demonstrating inhibitors to blood coagulation factors after repeat exposure to fibrin sealants. Am. J. Hematol. 64:59–63, 2000.

Key words: fibrin sealant; bovine thrombin; factor V; acquired inhibitor

INTRODUCTION

Fibrin sealants are becoming increasingly utilized as adjuncts in obtaining surgical hemostasis [1]. These products have wide applications in many areas such as trauma, cardiovascular, oncologic, vascular, and other surgical subspecialties [1]. Until recently, in the United States, the typical product was prepared exclusively by the blood bank of each institution and consisted of 10–15 mL of human cryoprecipitate combined with bovine thrombin. These components are initially kept separately in sterile syringes. When mixed, in the presence of factor XIII and calcium, fibrin is generated [2].

The use of fibrin sealants as historically prepared is fraught with many complications. The transmission of blood borne pathogens, including HIV, has been described [3]. Anaphylaxis to the bovine thrombin component has also been reported [4]. Coagulopathies including inhibitors to thrombin and factor V have been noted and are of concern [5–10].

Commercially prepared fibrin sealants have been available in Europe for more than 20 years. Recently similar products have been FDA approved in the United States for use in trauma and redo coronary artery bypass surgeries [1]. These new preparations are virally inactivated and use human thrombin, thus potentially limiting infectious disease transmission and obviating the development of inhibitors to coagulation factors. As the availability of these new preparations is not well known and their cost remains high, most centers still use locally prepared fibrin sealant. In addition topical bovine thrombin continues to be used frequently in dental and radiologic procedures to achieve hemostasis of oozing wounds.

We describe a patient who developed antibodies to both thrombin and factor V after repeat exposure to locally prepared fibrin sealant. Our patient required anticoagulation secondary to a recently placed mechanical aortic valve. The challenges in choosing proper anticoagulation, monitoring, and additional treatment are discussed.

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METHODS

Prothrombin time (PT), activated partial thromboplastin time (PTT), and thrombin time (TT) obtained at Shands Hospital, Gainesville, FL were performed by standard techniques utilizing an MLA-1600 C automatic coagulation analyzer (MLA Corp., Pleasantville, NY). The routine TT was performed by adding 0.1 mL of bovine thrombin (2 units/mL, Dade International Inc., Miami, FL) to 0.2 mL plasma that had been warmed to 37°C. Reptilase time was performed by following a standard procedure in a fibrometer [11]. Lupus anticoagulant activity was ascertained using the tissue thromboplastin inhibition test as described by Triplett et al. [15].

Factor II and factor V assays. Assays for specific coagulation factors were performed at Shands Hospital using a one-stage assay following the protocols provided by the manufacturers (MLA Corp., Pleasantville, NY, and Dade International Inc, Miami, FL). Each plasma sample was assayed at three dilutions (1:5, 1:10, and 1:20)

Bethesda assay. Inhibitor titers to factor V were detected and quantified as previously described by Kasper et al. [16]. Although originally developed for the evaluation of inhibitors to factor VIII, the same assay technique was used in the detection and quantification of the factor V inhibitor.

Enzyme-linked immunoassay for IgG antibodies to bovine and human thrombin. A 96-well Immunoplate (Nunc Inter-Med, Kamstrip, Denmark) was incubated with 50 μL of bovine or human thrombin (5 μg/mL) (Sigma Chemical, St. Louis, MO) diluted in 0.01 M sodium phosphate buffer, pH 7.2, containing 0.15 M NaCl and 0.02% NaN_3 (PBS-azide) at 4°C overnight. Subsequently, each well was washed three times with 200 μL of PBS-containing 0.2% Tween-20 (PBS-T) and then blocked with 100 μL of PBS-T containing 1% bovine serum albumin (blocking buffer) at 25°C for 30 min. After three more washes, 50 μL of plasma diluted 1:320 with blocking buffer were added into individual wells in duplicate and incubated at 25°C for 60 min. Wells were washed again four times, and 50 μL of goat anti-human IgG specific alkaline phosphatase conjugate (1:1000 dilution) (Sigma Chemical) were added. After incubation for 30 min at 25°C and four washes, 100 μL of *p*-nitrophenyl phosphate disodium (1 mg/mL prepared in 0.01 M sodium bicarbonate buffer, pH 9.6, containing 2 mM $MgCl_2$) was added and incubated at 37°C for 30 min. Absorbance at 405 nm was measured in a V_{max} ELISA plate reader (Molecular Device, Palo Alto, CA). Pooled normal plasma was included as a negative control in each assay. The variation between duplicate incubations was always less than 10%.

CASE REPORT

W.M. is a 66-year-old male transferred to Shands Hospital, Gainesville, FL on June 30, 1997 for evaluation of an acquired coagulopathy. The patient had undergone five-vessel coronary artery bypass grafting in May of 1993 and had been found to have critical aortic stenosis as well as progressive coronary artery disease. On June 11, 1997, he underwent an elective aortic valve replacement (CARBOMEDICS valve#21) and a one-vessel saphenous vein bypass graft. It was later determined that the patient received fibrin sealant during each procedure.

Preoperative coagulation studies for the 1997 procedure had been normal with a PT of 11.4 sec (11.1–13.1 sec, INR .96), PTT of 18.2 sec (21–32 sec.), and an Ivy Bleeding Time of 7.5 min. He had no prior personal or family history of bleeding.

The patient had an unremarkable post-operative course and was discharged 6 days later on warfarin 5 mg alternating with 7.5 mg daily. Two days after discharge, his INR was noted to be 2.7 and he was continued on the same dose of warfarin. However by post-operative day 13 the patient was noted to have a PT of 94 sec with an INR of 8.4. Overt bleeding did not occur. He was admitted to a local hospital and warfarin was discontinued. He was subsequently administered 17 units of fresh frozen plasma (FFP), and 25 mg of vitamin K subcutaneously over a 4-day period. Despite this treatment the laboratory values remained abnormal and on day 19, prior to transfer, the PT was markedly elevated.

The patient was transferred to Shands Hospital on post-operative day 20. Physical exam was essentially unremarkable and revealed no sites of bleeding or ecchymoses. Review of laboratory data from the referring hospital and at Shands Hospital (Table I) revealed a markedly prolonged PT, PTT, and thrombin time. The clinical situation, as well as clottable fibrinogen and fibrin split product values, was not consistent with disseminated intravascular coagulation (DIC). The thrombin time and PT did not correct on 1:1 dilution with equal volumes of normal plasma, suggesting an inhibitor to thrombin and possibly to another coagulation factor(s).

RESULTS

Characterization of Acquired Inhibitors

Thrombin time. The patient's thrombin time was initially >65 sec. At the time of transfer to our institution the thrombin time remained >60 sec and was elevated throughout the hospital stay. On follow-up visits 34, 42, and 49 days post re-exposure to fibrin sealant the thrombin time remained >60 sec. In March 1998, eight months after exposure to bovine thrombin the thrombin time had decreased, but remained prolonged at 30 sec.

TABLE I. Summary of Laboratory Results (July 1, 1997)*

		Normal values
PT	102	(9–12 sec)
INR	9.3	
PTT	126	(22–33 sec)
TT	>60	(10–15 sec)
PT 1:1 Dilution	67	(9–12 sec)
TT 1:1 Dilution	>60	(9–12 sec)
Lupus anticoagulant activity	Negative	(Negative)
Fibrinogen	633	(180–380 mg/dL)
Fibrin split products	<5	(<5 ug/mL)
Reptilase time	31.2	(14.7–21.7 sec)
Reptilase time 1:1 dilution	21.7	(14.7–21.7 sec)
Factor V (human)	<1.6%	(60–200%)
Factor V inhibitor	15 BU	(0–1 BU)
Factor II (human)	86%	(60–200%)

*Abbreviations: INR, international normalized ratio; PT, prothrombin time; PTT, partial thromboplastin time; TT, thrombin time; BU, Bethesda units.

Coagulation factors. Factor II activity was within normal limits at 86%. Factor V activity was severely reduced at <1.6%. An inhibitor against factor V was detected at 15 Bethesda units. Follow up factor V activity on day 41 after exposure to bovine thrombin had increased to 5%. It was at this time that the patient's PT/INR began to trend back toward normal values.

Factor VIII activity and von Willebrand factor assays revealed elevated levels (data not shown).

Ancillary coagulation studies. Mixing studies of patient plasma with equal values of normal pooled plasma did not correct the prolonged prothrombin and thrombin times. These studies remained prolonged greater than one month after exposure to fibrin sealant. The reptilase time was elevated at 31.2 sec, but it did correct when mixed with equal volumes of normal plasma.

A lupus-type anticoagulant could not be detected using the tissue thromboplastin inhibition assay.

Immunochemical assays. ELISA was used to identify antibodies against both bovine and human thrombin. Antibody titers were greater to bovine thrombin, but there was cross reactivity to human thrombin (Fig. 1). Anti-thrombin antibodies persisted for several months. Nine months after re-exposure to fibrin sealant anti-bovine and anti-human thrombin antibody activity remained detectable, although at much lower titers.

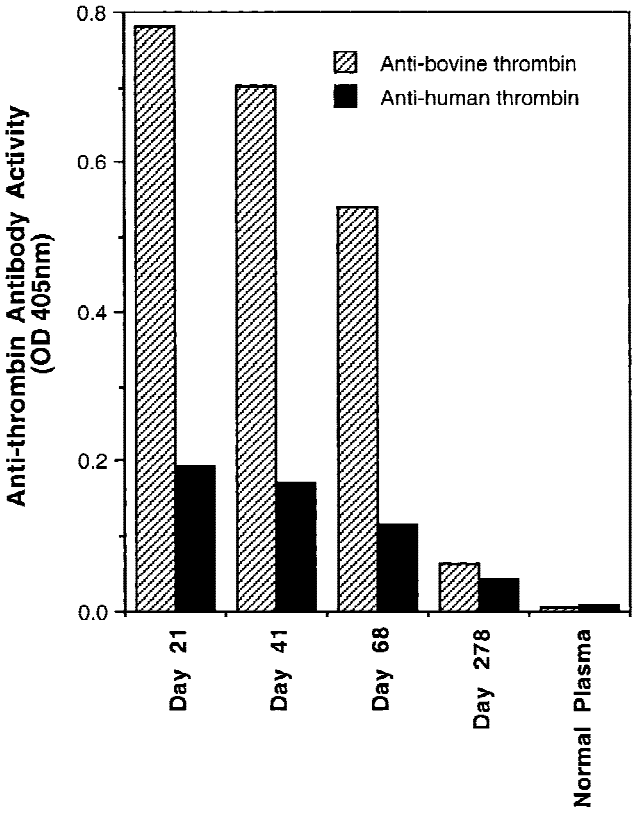

Fig. 1. Anti-bovine and anti-human thrombin antibody activity over time after exposure to fibrin sealant. Antibody activity in patient plasma is compared to normal donor plasma using an ELISA.

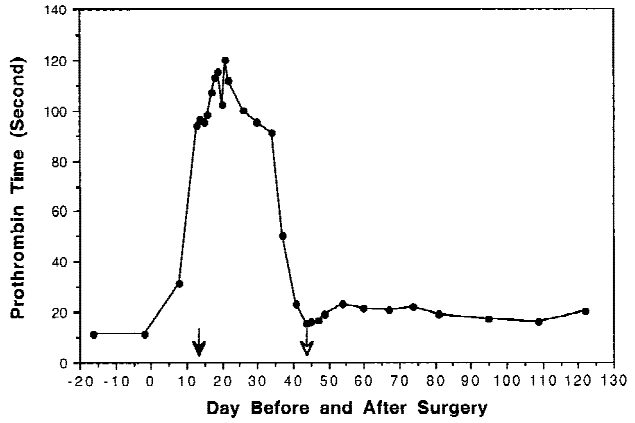

Fig. 2. Prothrombin time pre- and post-exposure to fibrin sealant. Solid arrow: patient admitted to local hospital. Warfarin was discontinued, FFP and vitamin K were administered. Open arrow: warfarin dosing resumed.

Clinical Course

Despite markedly elevated prothrombin, partial thromboplastin, and thrombin times our patient had no evidence of bleeding during his hospital course or during post-discharge follow-up. Due to the existing coagulopathy, it was initially decided not to proceed with pharmacologic anticoagulation. To monitor the function of the aortic valve, echocardiography was performed on July 2 and July 22, 1997. These studies demonstrated the valve to be functioning properly with no evidence of thrombus. The INR peaked at >13 approximately 1 month after re-exposure to fibrin sealant (Fig. 2). By 44 days after re-exposure the patient's INR had decreased to 1.6 and the factor V was measurable at 5%. The PT/INR was

considered subtherapeutic for prophylaxis of a mechanical valve, and it was decided to restart warfarin and titrate the dose to maintain an INR of 2.5–3.5. When the patient was evaluated in March 1998, eight months after his exposure to fibrin sealant, he denied any bleeding and was continuing warfarin for prophylactic anticoagulation of his mechanical aortic valve. He has been subsequently followed by his referring physician with no reported complications.

DISCUSSION

In the 1980s several reports appeared noting the identification of thrombin antibodies in post-surgical patients. Only in retrospect was it found that the majority of these patients had been exposed to fibrin sealant during their surgical procedures [13]. Co-immunization to bovine factor V has also been noted after exposure to fibrin sealant [5–7,9,10]. Zehnder and Leung have shown that crude preparations of bovine thrombin contain variable amounts of factor V, thus explaining the development of factor V antibodies in post-surgical patients who have received fibrin sealant [9].

La-Spada et al. reported that one-third to one-half of re-exposed patients develop these inhibitors [12]. Banninger et al. suggests that the development of antibodies to thrombin and factor V is strongly dependent on the type of operation and amount of fibrin sealant used [6]. In many reported cases the patients followed an uneventful clinical course with no serious bleeding despite having abnormal coagulation parameters [6,7,9,13]. However, major bleeding episodes [5,7,9,12] and death [5,12] have been described. The bleeding tendency may reflect the amount of cross reactivity to human factor V and thrombin [6]. As noted in our case, a decline in inhibitor titers to factor V, as reflected by normalization of the prothrombin time occurs spontaneously over many weeks, while the thrombin time remains prolonged for longer periods [5–7,10,13].

Most reports do not discuss the treatment of these acquired inhibitors, describe the bleeding episodes, or comment on the use of pharmacologic anticoagulation when indicated. Zehnder and Leung, however, describe the use of immunoglobulin, immunosuppressants, and low-dose chemotherapy in a patient with bleeding after fibrin glue exposure [9]. These manipulations failed to reverse the coagulopathy and control bleeding. Plasmapheresis was effective at providing transient correction of the coagulation tests and resolution of clinical bleeding. La-Spada et al. [12] describe a patient with lethal hemorrhage despite the use of plasmapheresis.

Ortel and colleagues suggest that despite having abnormal coagulation tests these patients should not be considered “autoanticoagulated” [5]. In fact, they have reported on a patient with a prosthetic mitral valve who developed septic thromboembolism originating from the valve surface despite the presence of an inhibitor to both factor V and thrombin. Unfortunately this patient also had significant clinical bleeding and did not respond to intravenous immunoglobulin.

Our patient demonstrated inhibitors to both human and bovine thrombin as well as factor V. An increase in the reptilase time was also observed and has been reported by others [5,10]. Chouhan et al. postulate that the elevation in reptilase time may be to be due to the formation of anti-fibrinogen antibodies [10].

Despite our patient’s prolonged laboratory values and identified inhibitors it was difficult to know if he was effectively anticoagulated in vivo. The risk of thromboembolism for patients with a mechanical heart valve who do not receive anticoagulation is estimated to be 8% per year [14]. Therefore, if our patient was not effectively anticoagulated for 2 months, a risk of thrombosis of his prosthetic valve greater than 1% might be expected. Even though we were in agreement with Ortel et al. [5] that this patient could not be considered “autoanticoagulated,” it was decided that his bleeding risk exceeded the risk of forming thrombus on the prosthetic aortic valve. Warfarin was withheld and serial surveillance echocardiograms were obtained to assess for thrombus. The prosthetic valve continued to function properly. Anticoagulation with warfarin was re-instituted as the PT began to approach the normal range.

The newly approved highly purified fibrin sealants produced by Baxter (Tissel VH) and Vitex contain virally inactivated human fibrinogen and human thrombin. If hospitals in the United States adopt the use of these new commercially derived fibrin sealants, complications from the blood bank derived product, including coagulopathy from antibodies against the clotting factors and transmission of lipid encapsulated virus can best be reduced by avoidance. Until such a time locally prepared products will likely continue to be used and complications, as discussed, should be anticipated. The management of these patients, especially those requiring effective anticoagulation continues to remain challenging.

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